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THE UNITED STATES OF AMERICA

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October 27, 2004

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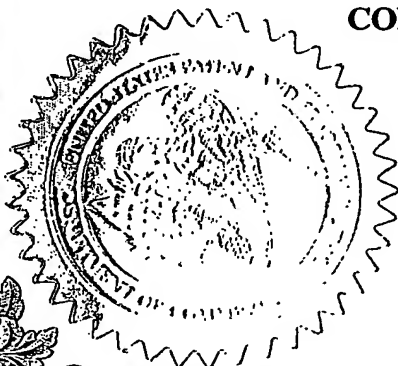
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PROVISIONAL APPLICATION COVER SHEET

APPROV

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).

Docket #: 5321-4P		Type a plus sign (+) inside this box →
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TITLE OF THE INVENTION (280 characters max)		
A Method of Collecting and Transporting Vaginal Discharge for Detection of Infectious Organisms		
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ENCLOSED APPLICATION PARTS (check all that apply)		
<input checked="" type="checkbox"/> Specification	Number of Pages <input type="checkbox"/>	<input type="checkbox"/> Other (specify):
<input checked="" type="checkbox"/> Drawing(s)	Number of Sheets <input type="checkbox"/>	
METHOD OF PAYMENT (check one)		
<input checked="" type="checkbox"/> A check is enclosed to cover the Provisional filing fees <input checked="" type="checkbox"/> If no check is enclosed or the enclosed check is insufficient - The Commissioner is hereby authorized to charge the filing fees or credit any overpayment to Deposit Acct. No. 03-2412.		PROVISIONAL FILING FEE AMOUNTS: \$ 80

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government

- ☒ No
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____
☐ Small Entity Status is claimed

Dated: December 26, 2002

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PROVISIONAL APPLICATION FILING ONLY

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Express Mail #EL798797444US

UNITED STATES

PROVISIONAL PATENT APPLICATION

**A Method of Collecting and Transporting Vaginal Discharge for Detection
of Infectious Organisms**

Inventor(s):

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FIELD OF INVENTION

The present invention relates to methods of collection of clinical samples of biological fluids or body discharge. In particular, the invention relates to methods of collection, storage and transportation of vaginal discharge so as to enable the detection of infectious disease agents by nucleic acid amplification or other methods of detection.

BACKGROUND OF THE INVENTION

The human body plays hosts to microorganisms, which can cause disease. Some of these enter the body through the female lower genital tract, infects the tissue and infects other individuals via the same portal through sexual contact or birth. One of these infectious disease agents is human papillomavirus (HPV). Scientific advances have elucidated the role of this virus in the causation of human cervical cancer. Over 99% of cervical cancer has detectable HPV generic material. The virus is both necessary and sufficient for neoplastic transformation.

The early detection of cervical cancer has saved many lives in the 50 years since the popularization of the PAP test. The PAP test is based on microscopic interpretation of stained cervical cells that are collected onto glass slides. Through the trained eyes of cytotechnologists and physicians, many early cervical neoplasia cases can be recognized before they turn into invasive cancer. However, the method has drawbacks, owing to failure of women to obtain a PAP test, sampling error, subjectivity inherent in any morphological interpretative tests and interpretative error. The sensitivity and specificity is therefore at an unsatisfactory level of 30-87%.

Since the association of HPV with cervical cancer is established, physicians have been trying various methods of detection of HPV from specimens obtained from the female lower genital tract. Methods of specimen collection have included biopsy, physician directed cervical scrape (similar to the process of obtaining a PAP test specimen), cervico-vaginal lavage and self-obtained specimens utilizing cervico-vaginal lavage, vaginal swab, vulval swab, self insertion and removal of vaginal tampon and urine collection.

All of these methods of obtaining clinical specimens have the serious drawback of requiring the patient to present herself to a clinic for the specimen collection, whether physician-directed or self-obtained. However, the major reasons why many women are still presenting with invasive cervical cancer without having a prior PAP test are the physiological barrier of denial, detest of strange environments and strangers, lack of time and other factors such as the unavailability of medical or screening facilities, particularly in developing countries. Attempts have also been made to collect air-dried clinical specimen including vulval swabs on toilet paper. However, there was no attempt to make use of vaginal discharge, which has collected over the course of the day on sanitary napkins, or on items for clothing, for the purpose of testing.

SUMMARY OF THE INVENTION

The invention provides methods for collection, storage and transportation of clinical specimen from the lower female genital tract collected on sanitary napkin for the purpose of testing.

According to the invention, the entire process of specimen collection is performed by the patient in the privacy of her own home using self-purchased slim sanitary napkin. In the

morning, the napkin is positioned between the underpants and the introitus and worn for at least 12 hours. At the end of the period, the patient removes the napkin and puts it into a zip-lock plastic bag. Before or after insertion into the bag, the napkin is dried by the gentle cool draft of a consumer-type electric hair drier for one minute. The zip-lock bag is then sealed, without decontamination, addition of preservative, dessicant or additional processing. Next the specimen is inserted into a regular envelope and mailed to the laboratory at the earliest convenience. The patient provides information that uniquely identifies the specimen, so that reporting can be made by mail, by telephone or other means. The identifying information need not be the patient's real name but must be unique and known only to the patient.

At the laboratory, the incoming specimens are opened, registered and tested. Testing consists of the steps of:

1. Individually opening the specimen bags under sterile conditions in a biohazard hood using decontaminated instruments.
2. Visual identification of the area of the napkin that contains visible stains.
3. Cutting out a piece of the stained napkin measuring 1 x 1 x 0.2 cm.
4. Insertion of the procured specimen into an Eppendorf tube for DNA extraction.
5. Measurement of DNA content by spectrometry after extraction to determine if dilution is required before subsequent amplification.
6. Dilution, if required of the extracted DNA.
7. Amplification of HPV DNA from the extracted DNA using two different reactions, employing primers GP5+/6+ and MY09/11.
8. Gel electrophoresis for the detection of HPV amplicons.

Example

Testing for cervical HPV infection is being investigated as a means of cervical cancer screening (1). Some investigators have tested for HPV on vaginal tampons (2) and have shown high patient acceptance (3) and concordance with physician-directed swab (4).

We tested the hypothesis of diagnosing genital HPV infection based on PCR of menstrual blood or vaginal discharge collected on sanitary napkins. We recruited a total of 10 patients, 7 with a pathological diagnosis of HPV infection, 2 with grade I cervical intraepithelial neoplasia and 1 with invasive squamous carcinoma of the cervix. Soiled intermenstrual or sanitary napkins containing menstrual blood were air-dried by a blower, placed in zip-lock plastic bags, and sent to us by regular mail. Small pieces of the napkins were cut out (1 cm x 1 cm x 1 mm) using sterile scissors for direct DNA extraction. We used the same amplification protocol (5) for both types of specimens, employing consensus primers GP5+ and GP6+ (biopsies and napkins) and in a different set of reactions (napkins only), primers MY09 and MY11. Gel electrophoresis was performed after 40 cycles of amplification. HPV was detected in the sanitary napkins in 100% (10/10) of cases. Nine specimens were positive using primer set GP5+/6+. One specimen (case 4) negative with GP5+/6+ tested positive with MY09/11.

Although the number of cases was small, the 100% sensitivity is encouraging. Significantly, even minimally soiled napkin (case 9) contained sufficient HPV DNA for detection. This study supported the hypothesis that HPV DNA remains detectable in vaginal discharge collected on sanitary napkins up to 7 weeks later and despite contamination by blood.

In populations where the prevalence of HPV infection and/or the rate of PAP testing are low, women can be targeted for PAP test or colposcopy by first testing for HPV status on soiled

sanitary napkins. While a negative test is not a complete reassurance, a positive HPV test justifies the trouble, embarrassment and time of the woman for a PAP test. Patients may even choose to be anonymous when testing for HPV. In this way, more patients may be screened by at least one of the two methods. Adoption of this paradigm shift may extend cervical cancer screening to a larger population of women.

Seven additional specimens of intermenstrual soiled napkins were tested with 100% positivity.

The cited references are herein incorporated by reference.

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